Investigation of *mec* A and (*tst-1*) Genes Among *Staphylococcus aureus*Isolated from Skin Infection in Al- Diwaniyah Iraq

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Abstract

In this study one handered and five samples were collected from skin infection in hospital Diwaniyah Dermatology Consultancy in Al-Diwaniyah governorate. from December 2018 to April 2019. All samples were cultured and Identified by using phenotyping tests. The results revealed that 41 isolates were *Staph. spp.* and 30 Isolates out of them Diagnosed *Staphylococcus aureus* by using (API staph). Virulence factors were explicated by using polymerase chain reaction (PCR) methicillin-resistant *staphylococcus aureus* (MRSA), were identified by detecting (*mec*A gene), which revealed that all skin infections were MRSA (100 %). Also, toxic shock syndrome toxin (TSST) were identified by using PCR to detect (*tst-1* gene 326 bp.), which revealed that 23 isolate were positive out of 30 isolate (76.33 %).

Keywords: *Investigation, mec A and (tst-1) Genes, Staphylococcus aureus*

Introduction

Many patients who are hospital reviewers suffer from Skin lesions and considered an important health problem [1] . *S.aureus* is one of the most important pathogens found in most places and also found naturally on human skin and is associated with a wide range of skin diseases The threat of this *S. aureus* is its morbidity, which is the factor of virulence produced [2] .The skin invasion of *S.aureus* either an essential internal source or external source [3] . The emergence of strains of *S.aureus* resistant to antibiotics such as MRSA is a global problem in clinical medicine [4] . Staphylococcal resistance to penicillin is mediated by producing penicillinase. Staphylococcal cassates chromosome mec (SCCmec) is a family of mobile genetic elements of *S. aureus* [5] . Resistance is given by the *mec*A gene by a protein associated with penicillin (PBP2a or PBP2), which has less affinity for act with β-lactams; allowing resistance to all β-lactam antibiotics, this mobile gene component has been acquired by different strains in separate gene transfer events, indicating that there is no common ancestor for the different MRSA strains. [6] . Toxic shock syndrome is a condition caused by this bacterial toxins, the primary mechanism involves the production of high-contrast antigens during topical Staphylococcal infection. The progression of the disease arises from superantigen toxin that activating multicellular T cells [7] .

Material and Method

The study was conducted on patients with skin lesion caused by *Staphylococcus aureus* in Al-Diwaniyah city / Iraq / 2019 and collected 105 samples included male and female. Patients (children, young and adults) range in age from 1 to 60 years in AL-Diwaniyah Teaching Hospital.

Isolation and Identification:

One handered and five samples were collected through a questionnaire (name ,sex, age ,) from skin lesion by transport media swab, then cultured on conventional media (blood agar, mannitol salt agar) This dishes were incubated at 37 °C for 18-24 and 48 h respectively, and diagnosed by biochemical testing. The diagnosis were isolates basis on phenotypic, catalase ,oxidase , and coagulase . These isolates were recognized based on common biochemical tests. (API Staph).that differentiate between species belonging to staphylococcal [8] .

antimicrobial susceptibility :

To performedThe antimicrobial susceptibility testing used the agar discs diffusion method as that described by [9]

Bacterial DNA Extraction :-

The extraction DNA has been done according to the manufacturer instructions.

Table (1) Primers used for *mec*A and *tst*-1genes amplification

Primer	Primer Oligonucleotide sequence, 5' to 3'	(bp)
mecA	F 5′- TGAGTTGAACCTGGTGAAGTT - 3′ R 5′-TGGTATGTGGAAGTTAGATTGG- 3′	855 bp
tst -1	F 5′-ACCCCTGTTCCCTTATCATC- 3′ R 5′-TTTTCAGTATTTGTAACGCC- 3′	326 bp

Table(2) Polymerase Chain Reaction :-

Step		Temperature	Time	Cycle
mecA	Initial denaturation	94.0C0	5 min	1
	Denaturation	94.0C0	1 min	35
	Annealing	57.0C0	2 min	
	Extension	72..0C0	1 min	
	Final Extension	72.0C0	5 min	
	Hold	4 0C	Forever	
Tst-1	Initial denaturation	94.0C0	5 min	1
	Denaturation	94.0C0	45 Sec	35
	Annealing	50.0C0	45Sec	
	Extension	72..0C0	45 seC	
	Final Extension	72.0C0	10 min	
	Hold	4 0C	Forever	

Table (3)Polymerase chain Reaction mastermix components

PCR Master mix	Volume
DNA template	5µL
Forward primer (10pmol/µL)	1.5µL
Reveres primer (10pmol/µL)	1.5µL
PCR water	4.5µL
Master mix	12.5 µL
Total volume	25 µL

To determine the presence of the desirable amplicon, electrophoresis that in 1.5% gel agarose stained with adding ethidium bromide, and the products that visualized using a UV transil-luminator.

Statistical analyses: the used , Chi square, were per-formed. A p-value less than 0.05 was considered statistically significant.

Results: In this study 30 isolates of *S.aureus* out of 105 isolates. were isolated from skin lesion such as abscesses, boils, pimples and folliculitis at Al-Diwaniyah Teaching Hospital, which was determined by phenotypic characteristics and via biochemical testing then confirmed , Ten different antibacterial were used against *S.aureus*. Resistance showed against some antibiotic, (30 isolates 100 %) resistant to each Ampicillin ,pencillin , cefoxatin, azthromycin, and trimethoprim , resistance was moderate to levofloxacin,chloramphenicol (12 isolates 40 %) clindamycin(15 isolates 50%) While lowest resistance to Amikcin (2 isolates 6.66) . But they are wholly active vancomycin (30isolates 100%). Polymerase chain reaction (PCR) used for amplification of *mec*A and *tst-1* genes. All the isolates from *Staphylococcus aureus* appeared to be carriers of *mec*A gene, which were 100 %by using specific primer 855 bp as figure (1) . while were results *tsst* that shown 23(76.66%) samples from the out of 30 isolates was positive by using specific primer 326 bp as figuer (2).

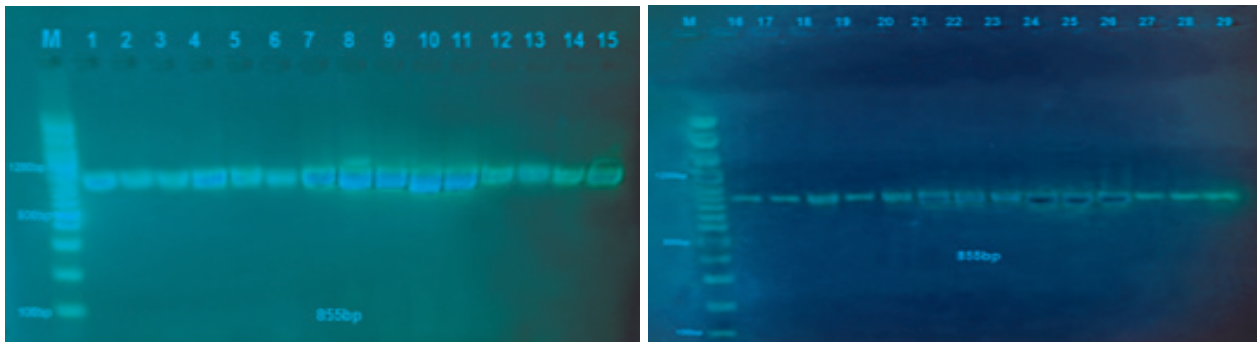

Figure (1) Ehidium bromide Gel electrophoresis (1.5%) of PCR of *MecA* amplicon product.(1-30) all positive for this *mecA* gene with amplicon size(855bp) in skin lesion isolates of *Staphylococcus aureus* for 1 hr. at 80 volts .

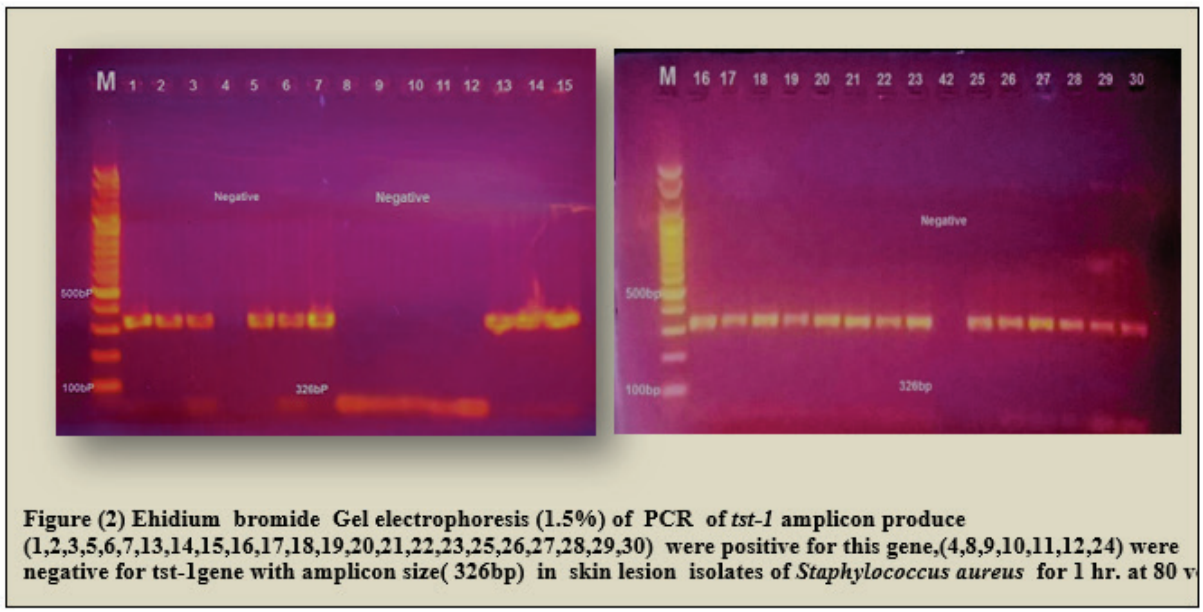

Figure (2) Ehidium bromide Gel electrophoresis (1.5%) of PCR of *tst-1* amplicon produce (1,2,3,5,6,7,13,14,15,16,17,18,19,20,21,22,23,25,26,27,28,29,30) were positive for this gene,(4,8,9,10,11,12,24) were negative for tst-1gene with amplicon size(326bp) in skin lesion isolates of *Staphylococcus aureus* for 1 hr. at 80 v

Discussion

The most commonly utilized antimicrobial substances in present clinical treatment procedures are β-lactams with intervention that includes inhibition of the last phase of the synthesis of bacterial cell-wall. These medicines have a smooth bactericidal with time-relied activities. They usually have excellent via-blood dissemination and little toxicity. Changes in the initial molecule resulted to the introduction of new substances with a wider antimicrobial action profile; however, in certain clinical environments, the use and effectiveness of beta-lactams are restricted because of increased bacterial resistance against those drugs [10] .

Resistance to β-lactams can be induced by various genes; however, the most well-known worldwide distributed gene is *mecA*. The staphylococcal cassette chromosome has a gene called *mecA* which codes for PBP that is responsible of resistance against β-lactam antibiotics [11] .

The action of the PBPs is manifested by their enzymatic activities due to inhibition-binding to β-lactams which results in destruction in the β-lactam chemical action properties. The PBP binding to β-lactams is occurred via the similarity present between the chemical structure of β-lactams and the chemical structure of the backbone of the sugar-amino acid that is an important part of the peptidoglycan [12] .

Resistance via the presence of *mec*A can be induced due to random use of antibiotics in various clinical conditions; however, resistance to certain antibiotics due to this gene were found to occur even with no previously exposure to those antibiotics [13] suggesting different means by which bacterial species can acquire such genetic resistance. One of the most important tools that bacteria employ to receive resistance is horizontal genetic transferr (HGT). Bitrus *et al.*, [14] have recognized that *mec*A gene can be transmitted from methicillin resistant *S. aureus* to susceptible strains via HGT, and this confirms that resistance can be present even with no previous history use of certain antibiotics. *S.aureus* can generate pathogenicity by employing various bacterial techniques; however, it can enhance potential damages to human body via harboring important genes such as toxic shock syndrome toxin *(tst-1)* gene. The work of this gene is recognized by its coding for an extracellular toxin that induce toxic shock syndrome. The health condition is characterized by the presence of severe symptoms such as fever, hypotension, rash, and malfunctioning of some body organs. In infants, TSS can generate Kawasaki syndrome, TSS-like exanthematous disease, and sudden infant death syndrome. The nowadays-reports refer to increasing the worldwide distribution of the *tst*-1 gene to even higher levels than the already known elevated spreading [15] . It has been found that this gene has genetic variations which might suggest different virulence levels [16] .

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Microbiology, Iraq and all experiments were carried out in accordance with approved guideline.

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